

# Anthropogenic Modifications to Estuaries Facilitate the Invasion of Non-Native Species

Enrique González-Ortegón <sup>1,\*</sup>  and Javier Moreno-Andrés <sup>2</sup> 

<sup>1</sup> Instituto de Ciencias Marinas de Andalucía, ICMAN-CSIC, Campus Universitario Río San Pedro s/n Pto. Real, 11519 Cádiz, Spain

<sup>2</sup> Department of Environmental Technologies, Faculty of Marine and Environmental Sciences, INMAR—Marine Research Institute, CEIMAR—International Campus of Excellence of the Sea, University of Cadiz, Campus Universitario de Puerto Real, 11510 Cádiz, Spain; javier.moreno@uca.es

\* Correspondence: e.gonzalez.ortegon@csic.es

**Abstract:** New observations of non-indigenous species (NIS) in coastal waters, such as the Gulf of Cadiz (Spain) have increased since 1980 and more or less exponentially in the last five years. Ballast water has become the most significant pathway for unintentional introductions of NIS into marine ecosystems. For example, the marine larvae of crustacean decapods that inhabit the water column could be transported in ballast water. Although elevated concentrations of metals are toxic to many marine organisms, some of them have evolved effective detoxification, or avoidance mechanisms making it possible to consider they have a superior ability to withstand exposures to these toxicants. In this text, we try to reinforce the hypothesis that anthropogenic modifications (such as chemical alterations and modified environments) benefit NIS with broad environmental tolerances. Taking these risks into account, a reinforcement of efficient Ballast Water Management Systems to respond to today's challenging environmental conditions is discussed.



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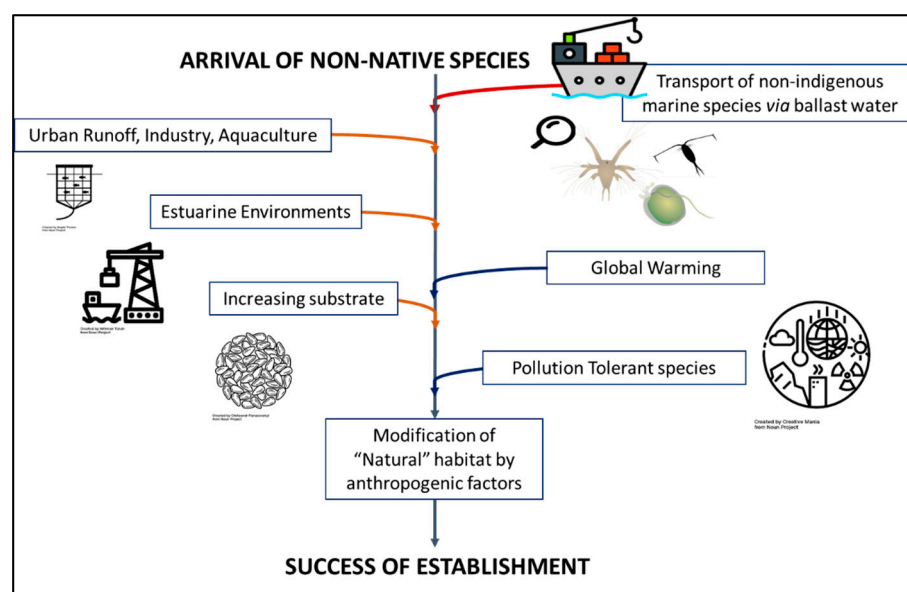
**Keywords:** exotic species; planktonic species; larvae; ballast water; gulf of Cadiz

## 1. Introduction: An Increase in the Arrival of Species

New worldwide observations of non-native invasive species (NIS) have increased over time, for instance in the Gulf of Cadiz (Spain), GoC, since 1980 and even more in the last five years [1]. Although the rate of new observations cannot be relied upon as an indicator of introduction rate, due to such factors as: sampling bias, occurrence of unexpected discoveries, lag time between introduction and discovery, and high variability in sampling effort, it does indicate increasing pressure on the GoC. The overall number of published articles about biological invasions appears, however, to be significantly biased towards new records, the hypothesis of the entrance of NIS in the recipient environment or the potential impacts in the native biological community; few studies analyzed the activities which they could determine species traits that affect the success of NIS post-arrival [2].

This communication is more an essay to reinforce the hypothesis that anthropogenic modifications (such as chemical alterations and modified environments) benefit NIS with broad environmental tolerances and how these invasive non-natives are often better suited to be able to survive in modified estuaries in which they are introduced (Figure 1). The extensive and accidental introduction of organisms in waters is a direct consequence of the intensity with which humans utilize this via for worldwide commerce [3]. Although, it is not possible to tease apart the invasion of species from 40 years ago due to the lack of records and long-term series, it is likely that the GoC present continues pressure at least since 1492, when Columbus sailed between this gulf and The Americas by wooden-hulled ships. From the 1500s, wooden-hulled ships could be colonized by marine organisms, which have been carried to many parts of the world, resulting in broad geographic distributions [4,5]. The use of steel as the primary ship-building material in vessels forced to use anti-fouling

paints which resulted in significant impacts to marine communities such as TBT or tri-butyl tin [6]. However, other constituents of antifouling paints to prevent the spread of species on vessel and boat hulls such as copper and zinc, which are common, seems to confer a competitive advantage on some non-indigenous marine invertebrates [7]. For instance, invasive bryozoans display a high tolerance to the toxic heavy metal copper [8] being a mechanism for the selection of toxicant tolerance in non-native species (see Section 3). Finally, although it is likely that the main transport of species could be associated to merchant vessels, we should take into account the situation for non-merchant vessels. For instance, recreational vessels are commonly transported to new locations by water without any attempt at cleaning.



**Figure 1.** Modifications of the habitat by anthropogenic factors (new artificial structures, or modification of the water quality) increase the chance of the establishment of non-native species.

Among the different ways that NIS species are introduced, ballast water has been considered one of the main vectors. These introductions might be facilitated due to the small pre-adult sizes (such as larval stages) and the particular risk of such transfers in ballast has been denoted for microorganisms [9,10]. In 2004, The International Maritime Organization (IMO) published the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWMC) that came into force in the year 2017. The main measure adopted was to establish a ballast water discharge standard through D-2 regulation, which was designed according to the organism's size: zooplankton ( $\geq 50 \mu\text{m}$ ) or phytoplankton species ( $\geq 10$ – $<50 \mu\text{m}$ ) and also established three independent bacterial indicators related to human health. However, some delays have occurred until today, which are related to the impossibility of implementing a validated Ballast Water Treatment System in the majority of vessels, among other factors. Despite many efforts being made to apply BWMC efficiently, the introduction of species via ballast water is still a global challenge [10].

Most of the exotic aquatic species are invertebrates such as decapod crustaceans, it is probably the fact that they have a larval phase, which inhabits the water column in the form of plankton, which ensures their transport in ballast water [11]. For instance, the initial invasion of the European green crab (*Carcinus maenas*) is thought to have taken place via the transport of larvae in ballast water [12], and they have also probably spread via anthropogenic intraregional transport [13]. The difficulty in identifying these early developmental stages (e.g., larvae and eggs) correctly is still a crucial issue, since these pre-adult stages are all common in ballast water [14,15]. However, recent studies have overcome this challenge with the use of advanced techniques, such as high throughput

sequencing, allowing the detection of pre-adult stages and other microorganisms [14]. For instance, Darling et al. 2020 found considerably higher ratios of arthropods (mainly copepods) or mollusks in ballast tanks [15]. In this way, the transport of larvae over long distances via ballast water could assure the transport of planktonic species or those species with a planktonic phase, although the success of their future establishment depends on biotic and abiotic factors upon arrival in the ecosystem.

This text focuses on how anthropogenic modifications influence the establishment of plankton (zooplankton ( $\geq 50 \mu\text{m}$ ) or phytoplankton species ( $10\text{--}50 \mu\text{m}$ )) and microorganisms at a location outside their native range (Figure. 1). With regard to anthropogenic modifications, we are referring to chemical alterations, modified environments or modifications of hydrological regimes in estuaries [2,16]. These scenarios will be discussed with some trends observed at the global scale; however, particular cases will be presented along the coastline of the south-west of Spain, concretely, the coastline of Cadiz. The coastal line of the GoC has suffered many anthropogenic transformations, with the presence of highly modified estuaries, important industrial activity and the presence of aquaculture and shellfish culture facilities. For instance, the Port of Algeciras, one of the biggest ports in Europe, is also present in this area.

## 2. The Suitability of an Estuarine Environment to Host Non-Native Species

The establishment of non-native species could be determined by the recurrence of an inflow of a species to a location outside its native range, and the biotic and abiotic conditions [17]. The wide variation in abiotic factors inside ballast tanks has also been reported [18] and although generally the viable number of phytoplankton and zooplankton drops within the holding time, it is not enough to meet the discharge standards.

Among the different aquatic ecosystems, estuaries show a wide variation in abiotic factors and most of the big estuaries are navigable and have big ports. These ports, servicing both inland waterways and oceanic shipping, are prone to inoculations of trans-oceanic biota and may, occasionally, promote a secondary spread of alien biota upstream. In this way, if the viable numbers of planktonic species are becoming resistant under variable abiotic conditions and are potentially discharged in these anthropogenic modified ecosystems, they might be more suitable to develop in these port areas, which are suitable scenarios for opportunistic species [9]. For instance, brackish-water macroinvertebrates are often introduced from ballast water to estuaries or coastal waters [19]. These ecosystems present a very wide saline gradient that could allow a variety of aquatic species to settle. In this way, 'invasiveness' is perceived to be particularly high in species with a broad environmental tolerance to both salinity and temperature [20].

For instance, although crustaceans show a specific salinity optimum for survival and development, which is related to their ecology, larvae of most euryhaline species in estuaries or even marine species of fish and macroinvertebrates with a strong estuary dependent juvenile phase, display an optimum that is within a wide range of around 15–25‰ [21,22]. The continued transport of planktonic species such as decapod crustacean larvae in ballast water would ensure the arrival of this group of species in plankton, which might explain the success of this group of species in coastal waters. Another example is the prymnesiphyte, *Prymnesium* sp., it is a euryhaline microalga, which is listed on the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae and has also been linked to ballast waters in the Bilbao Harbor (North-Spain), which is also an estuary [23]. Toxic blooms of this microalga can develop and can cause mortality in shellfish. In fact, such episodes have been detected in estuarine waters within the North Sea [24].

Thus, euryhaline species or estuarine dependent species may successfully invade a location outside their native range if they are transported to estuaries with a significant variability in salinity [22], these ecosystems are prone to invasion by exotic species.

### 3. Pollution-Tolerant Species: Species Likely to Be Invasive

Increasing commercial and recreational use, resulting in significant anthropogenic impacts on estuaries because of the rapid development of coastal watersheds, threaten the health of these ecosystems [25,26]. The excess of nutrient inputs (hypertrophication) in estuaries is usually linked to human activities within their drainage basin [27]. For instance, in the GoC, when nutrient concentrations of the Guadalquivir estuary were compared with those from other European estuaries, a nitrogen hypertrophication similar to that of the Westerschelde estuary (The Netherlands) [28] was observed [29]. In addition, chemical stressors such as trace metals, mainly associated with ports, or emerging compounds in highly modified estuaries, such as the Guadalquivir estuary or the Tinto-Odiel estuarine zone with major industrial activity [30] are a threat to the native populations and allow a favorable selection of the non-native species transported.

The majority of non-native marine species are transported in ballast water (mainly as larval stages) or as hull fouling as adult organisms [10,31], highly contaminated with metals [32]. In fact, certain amounts of heavy metals have been reported in ballast water [33,34]. More specific studies have detected a high metal concentration inside tanks when compared with the harbor water along the Persian Gulf [35]. A particular case is ballast sediments, which have been shown to be a reservoir of the resting stages of several invertebrates [36] but also an important area where certain amounts of metals accumulate [34]. Hulls and ballast water tanks are also painted with metal-based biocides leading to internal corrosion [37]. This is significant because NIS seem to acquire greater resistance to pollutants than native species [38] as a result of their stay in the hull. The transportation process may therefore select for metal tolerance, and the major contaminants in ports and harbors are metals [39].

The presence and effects of these emerging contaminants in these scenarios need further research: although some recent studies have detected antibiotic resistance genes in ballast waters [40,41], few experimental studies have combined the effects of emerging compounds or metals, together with environmental variability in terms of salinity and temperature, on survival and larval traits of marine and estuarine macroinvertebrates [42,43]. In addition, there is a lack of data on the effects of long-term low-dose exposure to pharmaceuticals and, especially, on the early developmental stages of marine and estuarine species [44,45], and even fewer studies take into account that organisms are exposed to these compounds in a context of environmental variability, such as estuaries [46,47], or the mode of action of those compounds which may differ depending on the species affected [48,49]. Overall, the contaminants tested did not show any clear effects on the survival and development of larvae exposed to environmental concentrations [42,43]. However, other studies on euryhaline species, which manipulated the access to food combined with emerging compounds considered sublethal under optimal food conditions, elicited lethal effects under conditions of food limitation [50]. Nevertheless, species that arrive at estuaries benefit from the rich feeding-grounds provided by these ecosystems, compared to other aquatic ecosystems. That is, overall, estuaries have a food web based on particulate and dissolved organic matter which includes bacteria and large mesozooplankton populations of detritivorous consumers, which are food for zooplankton predators such as juvenile fish and decapod crustaceans [29,51]. After exposure to contaminants and the associated stress, it is clear that NIS species benefit from the rich feeding-grounds provided by estuaries, increasing the likelihood of their successful introduction.

Coastal lines and estuaries, in many cases, have been completely modified and shipping selects for tolerant species thereby increasing the occurrence and densities of non-native species delivered in ballast water to contaminated locations. The majority of NIS are transported in ballast water or as hull-fouling organisms [31]. Metals are toxic to many marine organisms [52], but some organisms are able to withstand exposures to these toxicants [53]. This physiological stress would allow a selection in the transportation of those species, in such a way that those with efficient detoxification mechanisms or more resistant could survive their transportation better [2]. Thus, the coastal waters of the Gulf of

Cádiz, rich with chemical alterations, such as the Guadalquivir and Tinto-Odiel estuaries, could be a mechanism for the selection of toxicant tolerance in non-native species.

#### 4. Modifications to the Coastal Habitat: Increasing the Substrate of Non-Native Species

In addition to the modification of water quality and aquatic environment contamination, modified environments such as marinas or artificial structures on the coast increase retention of non-native species propagules and provide a substratum for their establishment [53–55]. Historically, the coastal line of the GoC has suffered several anthropogenic transformations such as the construction of four main harbors (in Algeciras, Cádiz, Seville and Huelva), reconstructed beaches (e.g., in Algeciras, Cádiz and Huelva) and wetlands (in the Guadalquivir river basin). The increasing transformation of natural to urbanized coastlines has promoted the establishment and spread of NIS [56]. Recently, a catastrophic bloom of *Rugulopterix okamurae* caused a significant impact throughout the Strait of Gibraltar (Spain). This species of algae had likely been introduced via ballast waters (although it cannot fully demonstrate), and its spread has increased, possibly due to global warming [57].

The global increase in anthropogenic activities results in hydrological modifications, creating environmental novelty and newly available artificial habitat [58,59].

Aquaculture has expanded greatly in the last few decades and deserves special attention. These activities can provide the substrate for potentially invasive species to settle and grow in the vicinity of areas with high maritime traffic, i.e., harbors, but they can also serve as a source of biofouling organisms that can attach to a new substrate, permitting them to be transported to a location where they can become invasive. These, together with the occurrence of other contaminants such as nutrients, pathogens or emerging contaminants may also be transferred to another area. For these reasons, harbors can be considered a point of transfer between aquaculture and shipping [60,61]. Some examples of this transference are the amoebic gill disease (*N. pemaquidensis*) [62] or the oyster parasite *B. ostreae* [63].

#### 5. Climate-Related Invasion of Non-Native Species

Many Mediterranean river discharges have declined more than the levels expected due to reductions in precipitation, and this is due to the flow regime of the water controlled by dams [64]. For instance, these effects have occurred clearly in the Guadiana and the Guadalquivir estuaries in the GoC [65,66]. In the case of the Guadalquivir estuary, the annual freshwater discharge since the construction of the Alcalá del Río dam (1930) has shown a significant long-term decreasing trend in dam discharges [64]. This anthropogenic reduction of freshwater discharge from Mediterranean rivers may create a different habitat, thus increasing the suitable habitat available for colonization by non-native species [67]. For instance, the Guadalquivir estuary and the adjacent salt marshes have recorded a high number of non-native species since the 1970s, even zooplankton species such as *Acartia tonsa* [68]. The interaction of water-usage practices and climate change anomalies has the potential to create events of new invasions, such as the co-occurrence of increased freshwater extraction and the resulting increased saline conditions in the San Francisco estuary that benefitted a non-indigenous zooplankton species (e.g., [69]).

Thus, introduction or invasions may be accelerated by global warming and enhanced by anthropogenic forces such as ballast water or aquaculture as previously discussed. Global warming has a direct effect on the proliferation of harmful algal blooms (HABs), which may also be promoted because of the contents of ballast water. These are of particular interest and concern because of their economic and health consequences [70]. Most of the related studies have been focused on the role of global warming in freshwater cyanobacterial invasion patterns [71]; however, further research is encouraged concerning marine HABs and the role of global warming in their spread. For instance, a HAB related paralytic shellfish poisoning event affected mussel aquaculture along the Cádiz coastline in the Algeciras Bay [72], which is an area that experiences high maritime traffic and industrial pressure.



Finally, climate warming will expand northward along the European Atlantic coast through the gulf of Cadiz and will benefit non-native species with wide environmental tolerances, as has already occurred with other macroinvertebrates [1].

## 6. Conclusions

Ballast water is an important vector for the dispersal of pre-adult stages and other microorganisms from different geographical areas that are not naturally connected. It has been observed that microscopic organisms and early development stages can develop and become invasive in over-exploited ecosystems that are highly influenced by anthropogenic perturbations, such as harbors and also modified environments, such as estuaries. Many efforts have been made to diminish the high impact of the NIS related to ballast water. However, due to advances in new techniques for species detection, the detection of many microscopic organisms is increasing, also including the early stages of the development of macroscopic organisms. In this scenario, a high abundance of microorganisms (including those below 10 µm in size) has been demonstrated in ballast waters and may have a significant impact on the receiving environment. Consequently, this fact should, perhaps, be taken into account in the discharge standards [9]. Additionally, it has also been demonstrated that the abiotic factors in ballast waters and receiving environments can vary widely and organisms in ballast waters are not fully decay by these abiotic factors [18]. In order to overcome these challenging conditions properly, the assessment and validation of ballast water treatment systems that can efficiently overcome these two factors (biotic and abiotic) is encouraged. Thus, it should involve a “worst case scenario” that could occur in real water conditions [18,73]. Additionally, the development of efficient ballast water management systems in these scenarios would also benefit some related industries, such as aquaculture, which is also, potentially, affected by possible spread of organisms resulting from an inadequate ballast water management [61,70].

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